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Effect of Paraquat on Organ Indices and Heamatology in *Clarias gariepinus* after Chronic Exposure

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Authors' contributions

All the authors participated in the carrying out of the experiment. The experiment was designed by author EOS who equally drafted the manuscript which was read and corrected by author EOA. Author EES executed the experiment in the laboratory. All the authors finally read and approved the final manuscript as presented.

Research Article

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ABSTRACT

The effect of paraquat on *Clarias gariepinus* (220.50 ± 15.33g mean weight and 30.43 ± 1.22cm mean total length) was examined by the assessment of the organ indices and haematological parameters after twenty one days of exposure. It was observed that paraquat caused decline in the fish condition, renatosomatic index, splenosomatic index and cardiosomatic index. However, there was an increase in the hepatosomatic index. There was decline in most of the blood parameters measured such as packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC) and RBC indices, though the decline were not significant (P≥0.05). Leucocrit fluctuated significantly (P≥0.05) in all the exposure concentrations. Platelets increased in level above the control value. White blood cells (WBC) declined slightly in all the exposure concentrations. The WBC counts, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) did not show any appreciable decline in levels, while lymphocytes showed slight decline in the lower concentrations and appreciated slightly in the highest concentration. Neutrophils increased in levels above that of the

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control value in all the concentrations except at the highest concentration where decline in level was observed.

Keywords: Haematology; organ indices; blood; paraquat; exposure.

1. INTRODUCTION

The extensive and intensive use of agrochemicals to meet the agricultural requirements of the population is inevitable and the illadvised (indiscriminate) use or misuse of these agrochemicals results in the poisoning of the biosystem [1]. Various pesticides used in aqua-agriculture to attack pest are known to adversely affect the biological system of organism [2,3]. Despite the fact that extensive use of pesticide have contributed immensely to agricultural productivity, there are reports of mass mortality of aquatic organisms after exposure to these toxicants, which in most cases are discharged accidentally [4].

Apart from the immediate signs of death that may be observed in the event of water pollution in cases of acute toxicity more commonly, organisms are subjected to long term effects (stress) resulting from chronic exposure which may also prove to be lethal to the organism or as toxicants to consumers of such organism [4,5,6]. However, reports on chronic toxicity of chemicals show that chronic levels can cause changes in behaviour [2], histopathology [3], enzymes and metabolites [7], feeding habits and feed conversion ratio [8] and haematological parameters [1,5].

Paraquat is a nitrogen based compound used as herbicide to control weeds on farms in the tropics. It was first synthesized in 1882, but its pesticidal property was discovered much later in 1959 [9]. It is in use the world over and can cause severe, acute and chronic poisoning when it is water borne [9] due to the fact that it readily dissolves and dissociates in water or aqueous solutions [10]. It is one of the most widely used herbicide globally and comes next to glyphosate. It is sold in over 130 countries for use on large and small farms, plantations and estates and in non weed control. It is quick acting, non selective herbicide, which destroys green plant tissue on contact and by translocation within the plant [11].

Paraquat is marketed by Zeneca as gramoxone for agricultural use in formulations ranging between 24-36% active ingredient. It is also used to control broad leaved weeds and grasses. It is increasingly used to destroy weeds in preparing land for planting in combination with no-till agricultural practices. Although it is toxic to fish, it is used as an aquatic herbicide [12]. Since its use is common in the tropics, therefore a good amount may be washed into streams, fish ponds, lakes and rivers through drifts and runoffs and may constitute chronic toxicity to the aquatic biota found in such environment.

This study is aimed at the determination of the effect of paraquat on the organ indices and haematology of the catfish *Clarias gariepinus* after chronic exposure.

2. MATERIALS AND METHODS

2.1 Source of Test Fish/ Acclimation of Fish

Clarias gariepinus (220.50 ± 15.33g mean weight and 30.43 ± 1.22cm mean total length) were bought from a private farm in Port Harcourt close to the University of Port Harcourt in Rivers State Nigeria. They were transported to the Chemistry Department laboratory of the Ignatius Ajuru University of Education, Port Harcourt. The fishes were acclimated to

laboratory conditions in individual tanks for seven days with bore hole water with 10 litre effective volume in a 30 litre plastic aquaria. The mouth of the aquaria were covered with 1mm nylon mesh to prevent escape of fish from the aquaria. The fish were fed with 31- 35% crude protein diet at 2% biomass during the acclimation and the experimental period.

2.2 Experimental Design and Preparation of Solutions

Completely randomized design was used in the experiment with five treatment levels and four replicates. The test solutions were prepared from a stock of 20g/L and was diluted to the desired concentrations (2.00, 4.00, 6.00 and 8.00mg/L) and a control. Fishes were fed during the experimental period as was in the acclimation.

2.3 Exposure of Fish to Paraquat

Four fishes were individually introduced into each aquarium containing the various concentrations of paraquat. The solution for the assay was renewed daily to maintain the concentrations.

2.4 Sample Collection

At the end of the experimental period (21 days), the weight of the fish were taken and blood samples were collected from the kidney of the fish by inserting 21G size needle between the anal fin and the orifice behind the anal fin. The collected blood was transferred by 5ml syringe into EDTA bottles for haematological analysis. The fish were then killed to remove the organs (liver, kidney, spleen and heart) which were weighed for organ index analysis.

2.5 Sample Analysis

Organ index and condition factor were calculated from the method described by Adams et al. [13].

$$\text{Organ index} = \frac{\text{Weight of organ} \times 100}{\text{Weight of fish}}$$

$$\text{Condition (K)} = \frac{\text{weight of fish} \times 100}{L^3}$$

Where L = length of fish.

Standard haematological procedures described by Blaxhall and Daisley [14] were employed in the determination of the various blood parameters. Packed cell volume (PCV) was determined in microhematocrit tubes after centrifuging for five minutes. Haemoglobin (Hb) was determined by the cyanomethaemoglobin method. Total white blood cell (WBC), red blood cell (RBC) and platelets were determined manually with a Neuauer hemacytometer using Natt-Herrick's solution as a diluent. For the differential counts (leucocrit, lymphocyte and neutrophils) were examined by dropping well mixed blood film on clean microscope slides and allowed to dry. The slides were fixed in methanol and the stained with Grumwall-Giemsa stain. The count of the cells was done noting the different cell type and their percentage occurrence. The value of the RBC indices was calculated according to Seiverd method [15].

$$\text{Mean corpuscular haemoglobin (MCH)} = \frac{\text{Haemoglobin} \times 10}{\text{RBC count}}$$

$$\text{Mean corpuscular haemoglobin concentration (MCHC)} = \frac{\text{Haemoglobin} \times 100}{\text{PCV}}$$

$$\text{Mean corpuscular volume} = \frac{\text{PCV}}{\text{RBC count}} \times 10$$

2.6 Statistical Analysis

The data obtained were subjected to analysis of variance (ANOVA) to determine if significant differences existed between the means in the parameters at different levels of contamination. Where differences existed, Duncan's Multiple Range Test (DMRT) was used to compare the difference between the means [16].

3. RESULTS

The physico-chemical parameters of the various treatment levels were not significantly different (P≤0.05), (Table1). The condition of the fish depreciated slightly in all the exposure concentrations. There was slight increase in the liver size, while the rest of the organs (kidney, heart and spleen) slightly decreased at various exposure concentrations when compared to the control (Table 2).

Packed cell volume (PCV) and haemoglobin (Hb) were declined below the value of the control. While PCV tend to show a concentration based decline, Hb did not show any definite pattern of decline. Leucocytes count was increased gradually in the lower concentrations but it declined gradually in the higher concentrations. Lymphocytes count declined in all the exposure concentrations except at the highest concentration (8.00mg/L) where slight increase was observed. There was an appreciable increase in platelets in all the exposure concentration above that of the control value. Mean corpuscular haemoglobin concentration (MCHC) did not show any significant (P≥0.05) variation from that of the control value. Mean corpuscular haemoglobin (MCH) did not show any noticeable change in value when compared to the control. Mean corpuscular volume (MCV) followed the same trend as MCHC and MCH. Red blood cells (RBC) and white blood cells (WBC) all declined in the exposure concentrations below that their respective control values. Neutrophils increased in value in all the exposure concentrations above that of the control except at 8.00mg/L (Table 3).

Table 1. Physico-chemical parameters of the exposure concentration of paraquat

Conc. of Paraquat (mg/L)	pH	Conductivity µS/cm	Dissolved Oxygen mg/l	Alkalinity mg/l	Hardness mg/l
0.00	5.95±2.10[a]	68.50±6.55[a]	7.26±3.31[a]	23.20±4.22[a]	8.50±0.25[a]
2.00	6.07±3.11[a]	72.88±8.89[a]	7.23±4.11[a]	25.85±6.61[a]	8.05±1.56[a]
4.00	5.89±2.00[a]	69.38±7.77[a]	7.22±3.22[a]	25.49±6.53[a]	7.60±2.80[a]
6.00	5.88±1.12[a]	73.25±9.90[a]	7.23±1.11[a]	24.77±4.47[a]	8.27±1.62[a]
8.00	6.15±2.33[a]	71.30±9.50[a]	7.35±1.51[a]	25.67±4.66[a]	8.27±1.56[a]

Means with the same superscript in the same column are not significantly Different (P≥0.05)

Table 2. Condition factor and organ indices of *Clarias gariepinus* exposed to various concentrations of paraquat for 21 days

Conc. of Paraquat (mg/L)	Initial condition K1	Final condition K2	HSI	RSI	SSI	CSI
0.00	0.73±0.02[a]	0.74±0.12[b]	0.55±0.05[b]	0.44±0.03[a]	0.09±0.00[a]	0.14±0.00[a]
2.00	0.88±0.01[a]	0.83±0.11[a]	0.65±0.04[b]	0.41±0.02[a]	0.08±0.01[a]	0.12±0.01[a]
4.00	0.65±0.00[a]	0.62±0.02[c]	0.68±0.01[a]	0.38±0.05[ab]	0.06±0.03[a]	0.11±0.02[a]
6.00	0.74±0.04[a]	0.71±0.03[b]	0.70±0.08[a]	0.40±0.01[a]	0.07±0.00[a]	0.13±0.00[a]
8.00	0.77±0.10[a]	0.72±0.10[b]	0.72±0.01[a]	0.36±0.00[b]	0.08±0.01[a]	0.10±0.00[a]

Hepatosomatic index (HSI), renasomatic index (RSI), splenosomatic index(SSI), cardiosomatic index (CSI). Means with the same superscript in the same column are not significantly different (P≥0.05)

Table 3. Haematological responses of catfish *Clarias gariepinus* exposed to various concentrations of paraquat for 21 days

Haematological Parameters	Concentration of paraquat (mg/L)				
	0.00	2.00	4.00	6.00	8.00
PCV (%)	15.50±1.23[a]	15.25±1.76[a]	10.34±4.62[a]	13.25±2.33[a]	11.38±0.43[a]
Hb (g/dl)	5.17±0.42[a]	5.083±0.54	3.42±1.53[a]	4.40±0.77[a]	3.79±1.48[a]
Leuco. (cells x 106/L)	1.50±0.58[ab]	2.00±0.82[ab]	3.00±1.00[a]	1.50±0.58[ab]	1.25±0.50[b]
Lympho. (%)	32.75±8.91[a]	27.50±9.19[a]	21.00±11.06[a]	26.25±12.69[a]	34.50±5.80[a]
Plat.(%)	18.88±9.00[a]	45.00±25.14[a]	25.00±14.43[a]	32.88±11.51[a]	26.63±10.93[a]
MCHC (g/dl)	16.50±0.00[a]	16.38±0.25[a]	16.34±0.29[a]	16.38±0.25[a]	16.25±0.29[a]
MCH (pg)	16.00±0.00[a]	15.88±0.25[a]	15.67±0.58[a]	15.75±0.29[a]	15.75±0.50[a]
MCV (fl)	48.25±0.29[a]	48.00±0.71[a]	47.00±1.73[a]	48.13±0.25[a]	47.50±0.71[a]
RBC (cells x 109/L)	3.20±0.24[a]	3.15±0.35[a]	2.17±0.92[a]	2.75±0.47[a]	2.76±0.68[a]
WBC(cells x 106/L)	11.82±4.63[a]	10.17±7.33[a]	10.85±6.40[a]	10.23±4.43[a]	9.23±4.36[a]
Neutro (%)	16.00±6.57[a]	18.38±8.19[a]	29.00±11.56[a]	23.75±12.69[a]	15.50±5.80[a]

Packed cell volume (PCV), heamoglobin (Hb), leucocrit (leuco.), lymphocyte (lympho.), platelets (plat.), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), red blood cells (RBC), white blood cell (WBC), neutrophils (neutro.). Means with the same superscript in the same row are not significantly different (P≥0.05).

4. DISCUSSION

It has been established from studies that sublethal concentrations of toxicants in aquatic environment often results in several physiological dysfunctions instead of outright mortality of the organism [17] .

The general health status of the fish which is known as organ indices is used to assess the physiological condition or well being of the fish and to determine the severity or damage caused by the toxicant on the fish. In this study, there was decreased condition of the fish similar to those observed in Clarias gariepinus exposed to *Lepidagathis alopecuroides* [18], *Nicotiana tobaccum* [19] and monocrotophos [8] and when *Labeo rohita* was exposed to monocrotophos and and endosulphan [20]. Decrease in condition can result from incomplete elimination of the toxic chemical from the fish organs, thereby interfering with the normal metabolism of the fish and therefore lead to inefficient production and utilization of protein and carbohydrate in the tissues of the fish [21,22] .

The increase in liver size (hepatosomatic index) in the presence of toxicants as was observed in this study, possibly may have resulted from increase in the production of

endoplasmic reticulum for protein synthesis in the liver tissue of the fish during toxicant induced stress [23] or due to cell enlargement or cell multiplication [24]. The reduction in renatosomatic, splenosomatic and cardiosomatic indices observed indicated decrease in the size of the kidney, spleen and the heart of the fish. These organs are associated with blood production and circulation, therefore the development of new blood cells, immunological interaction, blood filtration and circulation will be seriously affected [18,23]. Hence, decrease in size may be a pathological condition which indicates destruction of red blood cells.

Decrease in PCV, Hb, RBC and RBC parameters (MCH, MCHC and MCV) observed in other studies when fishes are exposed to toxicants (Omoniyi et al. [1,19,22] which conforms to the ones observed in this study. The decrease in the above named parameters suggests that paraquat induces changes which indicate a decrease in haematopoiesis, followed by anaemia induction in the fish under test [26]. According to Sampath et al. [27], decrease in these blood parameters is attributed to haemolysis which results in haemodilution to reduce the effect of the toxicant in the tissue system. This can also influence the increased rate of the Hb destruction or decrease in its production or synthesis [28]. Decrease in PCV is an indication of shrinkage of cell size and or decrease in the number of cells [29]. The value of Hb in the blood is an indication of the amount of oxygen available to the tissues of the organism, therefore decrease in Hb levels will impair oxygen supply to the various tissues resulting in low energy production and slow metabolic rate [29,30]. If this condition is allowed for a long time in the presence of the toxicant, the fish will develop blood dyscrasis and degeneration of erythrocytes.

Decrease in RBC and its parameters is attributed to decreased erythropoietic activity which in most vertebrates, including fishes is regulated by the erythropoietin produced in the kidney [5, 31]. Inducing haemopoietic stem cells to differentiate erythroblasts, which form RBCs are caused by erythropoietin promoted erythropoiesis [32] which is formed in the kidney, thus suggesting low blood oxygen in the tissues. Decrease in RBC and its parameters (MCH, MCHC and MCV) imply the malfunctioning of the kidney and the spleen and also the organs responsible for blood production in the fish. Platelets increased in levels in the fish and this mechanism in the fish is to dilute the effect of the toxicant on the fish. Increased rate of production of platelets by the head kidney due to toxicant effect helped to strengthen the WBC in carrying out its function of combating toxicants or invaders and also in blood clothing in the fish in the event of injury caused by the toxicant [18,33]. WBC and WBC counts (neutrophils, lymphocytes and leucocrit) are completely involved in the regulation of immunological functions of organisms and therefore prolong exposure of *Clarias gariepinus* to paraquat may lead to immunological dysfunctions or deficiency [5]. Decrease and increase in leucocrit and WBC counts arises from decrease or increase in the number of lymphocytes and thrombocytes [34,35]. However, decrease in the parameters results from impaired production capacity of cells which culminates in the decrease of cellular lifespan of cells, lymphopoenia and necrosis of leucopoietic tissues [36]. This condition can also lead to the destruction of cortiscosteroid hormones and the accumulation of lymphocytes in lymphoid tissues [34,37].

5. CONCLUSION

The toxicant (paraquat) caused a general disorder in the organs and haematology of the fish which is an indication that it is toxic to the fish at sublethal concentrations and so can cause death after prolonged exposure. Therefore its use should be adequately restricted to avoid contact with the aquatic environment.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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